

Distribution of metals and trace elements in adult and juvenile penguins from the Antarctic Peninsula area

Silvia Jerez · Miguel Motas · Jesús Benzal · Julia Diaz ·
Virginia Vidal · Verónica D'Amico · Andrés Barbosa

Abstract The presence of metals in the Antarctic environment is principally a natural phenomenon caused by geochemical characteristics of the region, although some anthropogenic activities can increase these natural levels. Antarctic penguins present several of the characteristics of useful sentinels of pollution in Antarctica such as they are long-lived species situated at the top of food web. The concentrations of Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Cd, and Pb were determined by inductively coupled plasma–mass spectrometry in samples of liver, kidney, muscle, bone, feather, and stomach contents of gentoo, chinstrap, and Adélie penguin (12 adults, five juveniles) from carcasses of naturally dead individuals collected opportunistically in the Antarctic Peninsula area. The obtained results showed that accumulation and magnification of several elements can be occurring, so that Cd and Se reached levels potentially toxic in some specimens. The presence of human activities

seems to be increasing the presence of toxic metals such as Mn, Cr, Ni, or Pb in penguins.

Keywords Metals · Trace elements · Pollution · Antarctic penguins · Antarctic Peninsula

Introduction

The presence of metals in the Antarctic environment is a natural phenomenon caused by the geochemical characteristics of the different areas in Antarctica (Andrade et al. 2001; Sánchez-Hernández 2000), although several anthropogenic sources (oil spills, paints, open field garbage burning, or fuel combustion) contribute to increase these natural levels (Claridge et al. 1995; Curtosi et al. 2010; dos Santos et al. 2005; Poblet et al. 1997; Vodopivec and Curtosi 1998).

S. Jerez (✉) · M. Motas
Área de Toxicología, Facultad de Veterinaria,
Universidad de Murcia,
Campus de Espinardo,
30100 Murcia, Spain
e-mail: silviajerez@um.es

J. Benzal · A. Barbosa
Departamento de Ecología Funcional y Evolutiva,
Estación Experimental de Zonas Áridas, CSIC,
Carretera de Sacramento s/n, La Cañada de San Urbano,
04120 Almería, Spain

J. Diaz
Centro de Estudios Parasitológicos y de Vectores
(CCT La Plata-CONICET-UNLP),
No 584,
1900, La Plata, Argentina

V. Vidal
Departamento de Sanidad Animal,
Facultad de Veterinaria, Universidad de Murcia,
Campus de Espinardo,
30100 Murcia, Spain

V. D'Amico
Centro Nacional Patagónico (CENPAT-CONICET),
Boulevard Brown 2825,
Puerto Madryn, Chubut, Argentina

A. Barbosa
Departamento de Ecología Evolutiva,
Museo Nacional de Ciencias Naturales, CSIC,
C/ José Gutiérrez Abascal 2,
28006 Madrid, Spain

Although the Protocol on Environmental Protection to the Antarctic Treaty (the Madrid Protocol of 1991) initiated a stage of regulation and control of the Antarctic activities, before its implementation many human activities were carried out without any consideration for the environmental health of this region (Curtosi et al. 2010). In addition, the recent human activity, such as research and an increase in tourism in the northern area of the Antarctic Peninsula (6,704 tourists and 59 voyages in 1992–1993 and 36,875 tourists and 239 voyages in 2009–2010, IAATO 2010) and its associated activities such as plane and ship trips, could have a significant effect on the accumulation of metals in the Antarctic biota. In this context, it has been observed that the levels of some trace metals are higher in Antarctic areas where an important human presence exists in comparison to more untouched areas (Jerez et al. 2011).

In this way, Antarctic seabirds may be useful indicators of regional environmental health, presenting several advantages for pollution monitoring in comparison to other organisms: They are top predators and long-lived species (biomagnification and bioaccumulation phenomena can occur), present wide distribution ranges with abundant populations, and integrate contamination over time and space (Burger and Gochfeld 2004; Walsh 1990). The main disadvantage in Antarctica is the difficulty to get large sample sizes as it is not allowed under the Antarctic Treaty to collect living individuals. Moreover, ethical standards recommend the use of non-invasive methods for sampling as an alternative to capture and sacrifice organisms. For this reason, several authors have used feathers, excreta, or eggs to study the abundance of metals and trace elements in Antarctic seabirds (e.g., Ancora et al. 2002; Bargagli et al. 1998; Jerez et al. 2011; Kureishy et al. 1993; Metcheva et al. 2006; Scheifler et al. 2005; Stewart et al. 1997; Sun and Xie 2001). However, valuable data on levels of these elements in internal tissues are still scarce (Bargagli et al. 1998; De Moreno et al. 1997; Honda et al. 1986; Norheim 1987; Nygard et al. 2001; Smichowski et al. 2006; Szefer et al. 1993a, b).

This study aims to increase the information on this issue investigating the concentrations of metals and trace elements in internal tissues and stomach contents of three species of Antarctic penguins (gentoo penguin—*Pygoscelis papua*, chinstrap penguin—*Pygoscelis antarctica* and Adélie penguin—*Pygoscelis adeliae*), identifying target organs for metal accumulation, studying the possible existence of biomagnification phenomena, and verifying if elevated or toxic levels are reached in penguin tissues.

Materials and methods

Penguin carcasses from naturally dead individuals (12 adults and five juveniles, see Table 1) were opportunistically collected

Table 1 Studied specimens

Specie	Location	Age	Samples
<i>P. papua</i>	King George Island	Adult	L, K, M, B, SC
<i>P. papua</i>	King George Island	Adult	L, K, M, B, F, SC
<i>P. papua</i>	King George Island	Adult	L, K, M, F
<i>P. antarctica</i>	King George Island	Adult	L, K, M, B, F, SC
<i>P. antarctica</i>	King George Island	Adult	L, K, M, B, F, SC
<i>P. adeliae</i>	King George Island	Adult	L, K, M, B, F, SC
<i>P. antarctica</i>	Deception Island	Adult	L, K, M, B
<i>P. antarctica</i>	Deception Island	Adult	L, K, M, B, F
<i>P. antarctica</i>	Deception Island	Adult	L, K, M, B, F, SC
<i>P. antarctica</i>	Deception Island	Adult	L, K, M, B, F
<i>P. adeliae</i>	Avian Island	Adult	L, K, M, B, F, SC
<i>P. adeliae</i>	Avian Island	Adult	L, K, M, F, SC
<i>P. papua</i>	King George Island	Juvenile	L, K, M, F, SC
<i>P. papua</i>	King George Island	Juvenile	L, K, M, F, SC
<i>P. papua</i>	King George Island	Juvenile	L, K, M, F, SC
<i>P. papua</i>	King George Island	Juvenile	L, K, M, F, SC
<i>P. adeliae</i>	King George Island	Juvenile	L, K, M, F

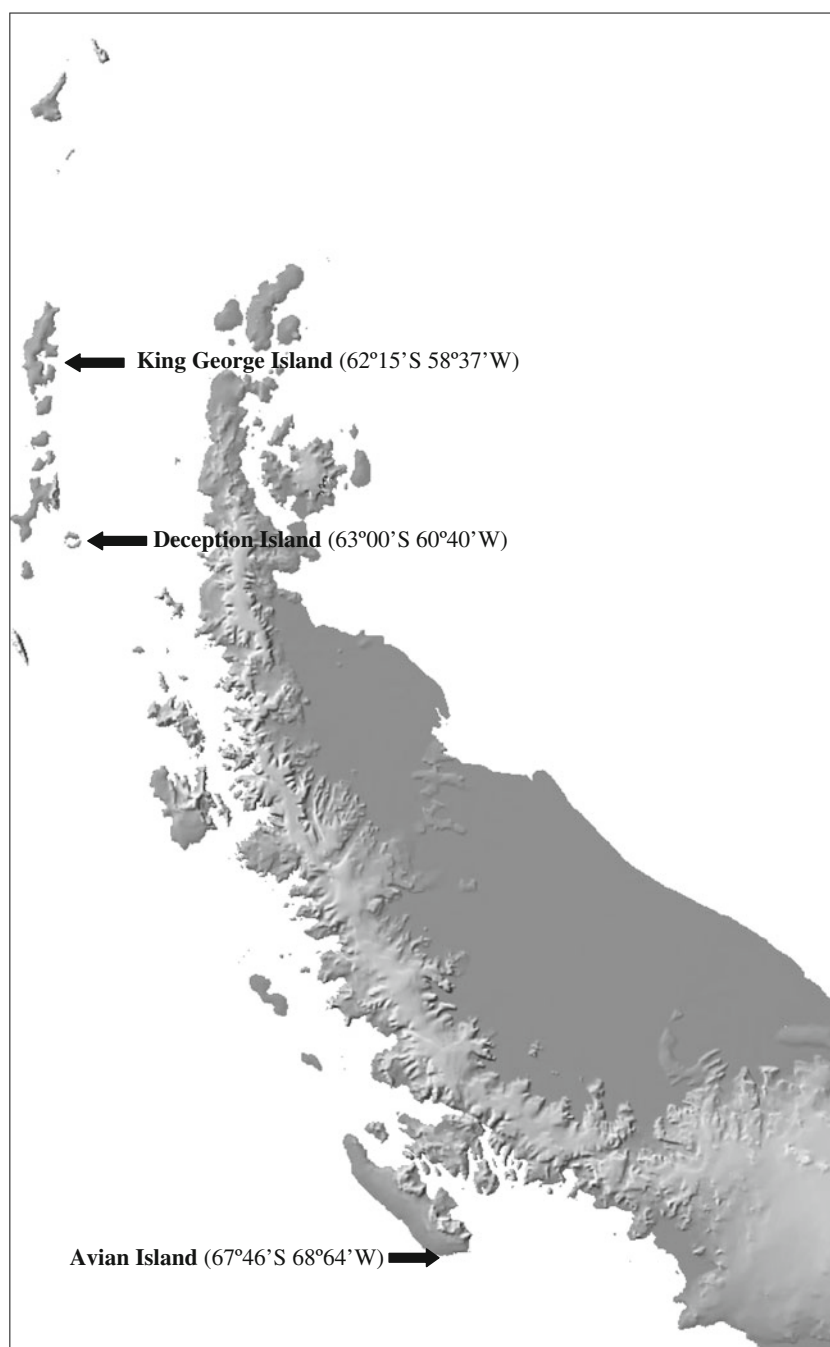
L liver, K kidney, M muscle, B bone, F feather, SC stomach content

during austral summers (from December to February) from 2007 to 2010 in different locations of the Antarctic Peninsula area, ranging from 62°15'S 58°37'W to 67°46'S 68°64'W (see Fig. 1). A specimen was considered as a juvenile when it has nearly finished the shedding of down feathers into adult plumage. Samples of liver ($n=17$), kidney ($n=17$), muscle ($n=17$), bone ($n=10$), feather ($n=15$), and stomach content (composed mainly of krill) ($n=12$) were taken by necropsies of the penguin carcasses and frozen individually in polyethylene bags.

The analytical method used in this study was that of Jerez et al. (2010) with minor modification. Before analysis, penguin tissues were rinsed with Milli-Q water, and all the samples were homogenized and dried at 75–80 °C till constant weight. Between 0.1236 and 0.4324 g of the material, according to availability, were subjected to microwave digestion with HNO₃ (65 %), H₂O₂ (30 %), and H₂O (proportion 5:2:3). The elements Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Cd, and Pb were determined by mass spectrometry with inductively coupled plasma (ICP-MS Thermo-Optek Serio X7). All of the reagents used were Suprapur (Merck), and the water was double-distilled and deionized (Milli-Q system, Millipore, USA). The analytical precision was verified by use of blanks every five samples, initial calibration standards, and certified reference materials (DORM-2 and DOLT-2; Table 2).

According to Smith et al. (2007), values below instrumental detection limits were predicted from expected normal scores when more than 50 % of all samples showed detectable levels within each data set. If not, detection limit values were assigned.

Fig. 1 Sampling sites



Statistical analyses were conducted using SPSS version 15.0. Non-parametric statistical methodologies were used because of the assumptions of normality and homoscedasticity were not met even after the log transformation of the data. Statistically significant differences in metal accumulation in penguin internal tissues, feathers, and stomach contents and differences in metal concentrations between juvenile and adult individuals were assessed by using Kruskal–Wallis and Mann–Whitney *U* tests. Post hoc tests were carried out for Kruskal–Wallis analyses (least significant difference between mean ranks). Spearman rank correlation coefficients were calculated

between pairs of elements. A *p* value less than 0.05 was considered to indicate statistical significance. Results are presented as mean±standard deviation in micrograms per gram dry weight. Small sample sizes preclude statistical analyses for inter-specific and inter-population comparisons.

Results and discussion

Different accumulation patterns were observed in penguin organs and tissues (Tables 3 and 4). Cd levels showed that

Table 2 Detection limit values (nanograms per gram), reference material values (micrograms per gram), and percentage of recovery obtained

Element	Detection limits	DORM-2	% recovery	DOLT-2	% recovery
Al	3.88	10.90±1.70	80.20	25.20±2.40	97.43
As	0.20	18.00±1.10	93.44	16.60±1.10	95.39
Cd	0.10	0.043±0.008	93.02	20.80±0.50	97.34
Cr	0.20	34.70±5.50	85.63	0.37±0.08	90.81
Cu	0.80	2.34±0.16	94.36	25.80±1.10	102.13
Fe	1.70	142.00±10.00	97.84	1,103.00±47.00	89.97
Pb	0.80	0.065±0.007	110.70	0.22±0.02	89.15
Mn	0.40	3.66±0.34	103.93	6.88±0.56	86.44
Ni	0.40	4.64±0.26	84.87	2.14±0.28	107.00
Se	0.70	19.40±3.10	93.36	0.20±0.02	94.54
Zn	2.70	1.40±0.09	85.41	6.06±0.49	94.89

this toxic metal was mainly accumulated in the kidney and liver of penguins in comparison to the rest of analyzed tissues ($p<0.0001$), which is a common pattern previously described for several organisms (e.g., Burger 2008; Koizumi et al. 2008; Włostowski et al. 2010). The low ratio we observed between Cd concentrations in hepatic and renal tissues (ratio $Cd_{liver/kidney}=0.08$) was indicative of a chronic exposure to relatively high levels of this metal. Besides, penguins accumulated Cd during their life time (Cd levels in the kidney, muscle, and feather in adults were higher than those detected in juveniles, $p<0.05$), giving rise to elevated Cd levels in kidney of adult specimens, even above the tentative toxicity thresholds described for birds (Furness 1996; Larison et al. 2000; Rodrigue et al. 2007; Stout et al. 2002) (see Fig. 2). Our results also showed clear signs of biomagnification: Cd concentration in penguin kidneys was 314.43 times higher than the concentration detected in their stomach contents composed of krill, the main penguins' prey ($p<0.0001$).

The accumulation of toxic concentrations of Cd can cause several adverse effects on bird health: renal tubular and testicular damage, disorder in calcium balance and skeletal integrity, reduction of food intake and growth rate, diminution in egg laying, egg shell thinning or behavior alterations, among others (Burger 2008; Furness 1996; Larison et al. 2000; Rodrigue et al. 2007). However, marine birds seem to be less vulnerable to exposure to high Cd levels than other wild organisms and birds (Burger 2008; Furness 1996).

Local volcanism is important in the study area (Thomson et al. 2001), and it can be an important natural source of metals such as Cd for organisms (Burger and Gochfeld 2004). This volcanic activity could be the responsible, at least partially, of the observed great differences on Cd concentration among the studied rookeries (see Tables 3 and 4). Other natural phenomena have been proposed as important Cd sources in Polar Regions, for example, upwelling of Cd-rich waters and algal bloom (Bargagli et al.

1996). But also anthropogenic sources at local and global scale (smelters, batteries, paints, corrosive coatings, plastic stabilizers, fertilizers, etc.) can bring about an increase of Cd levels in Antarctica since this metal can be long-range transported atmospherically bound to fine particles (Burger 2008; Furness 1996; McLaughlin et al. 1996).

Se was mainly accumulated in the liver and kidney, whereas Zn was mainly accumulated in the liver, kidney, and bone ($p<0.0001$; Tables 3 and 4) as it occurs in other seabirds (Kim et al. 2009; Ribeiro et al. 2009). As in the case of Cd, Se and Zn levels found in several penguin internal tissues were higher than those detected in their stomach contents (4.99 and 3.97 times higher in the kidney, respectively, $p<0.0001$). These high Se and Zn levels and the observed positive correlations between Se–Cd and Zn–Cd in penguin tissues (see Table 5 and Fig. 2) can be related to the detoxifying role that these essential elements play against Cd toxicity (e.g. Jerez et al. 2011; Norheim 1987): High Se and Zn levels can be protecting penguins, at least partially, against the toxic effects prompted by the exposure to elevated Cd levels.

We also observed differences among adult and juvenile specimens in the kidney, muscle, and feather ($p<0.05$, Tables 3 and 4) that point out that Se was accumulated in penguins during their life cycle. Although Se is an essential element and can play a protection role against Cd toxicity, Se accumulation can also be causing adverse effects on penguins' health, such as behavioral abnormalities or reproductive deficits (Eisler 1985; Heinz 1996; Ohlendorf 1989). We observed that 47.06 % of the studied specimens showed higher Se levels in liver than the toxicity threshold established by Lemley (1993) for aquatic birds ($10 \mu g g^{-1} d.w.$), although the interaction of Se with other trace elements may modify its toxicity (Heinz 1996). These high Se levels detected in penguins could be related to the high Se levels detected in their stomach contents; the 58.33 % of these samples contained Se concentrations potentially toxic for penguins (more than $3 \mu g g^{-1} d.w.$ in preys is considered as toxic for aquatic birds, Lemley 1993).

Table 3 Concentrations of trace elements (micrograms per gram dry weight) in adult Antarctic penguins

Samples	Specie (Location)	Al	n	Cr	n	Mn	n	Fe	n	Ni	n	Cu	n	Zn	n	As	n	Se	n	Cd	n	Pb	n
Liver	<i>P. papua</i> ^b	2.19±0.52	0	0.19±0.13	0	7.71±6.34	0	2,869.46±3,051.88	0	0.01±0.004	1	102.57±155.93	0	112.56±72.69	0	1.01±0.90	0	6.47±0.82	0	1.05±1.43	0	0.10±0.07	0
	<i>P. antarctica</i> ^b	1.00±0.14	0	0.26±0.02	0	14.76±4.17	0	4,002.21±2,744.67	0	0.02±0.02	0	24.26±11.18	0	330.34±293.26	0	0.37±0.36	0	7.18±0.38	0	0.16±0.08	0	0.05±0.01	0
	<i>P. adeliae</i> ^b	4.19	0	0.76	0	8.58	0	2,040.44	0	0.01	0	10.91	0	136.30	0	1.20	0	8.61	0	4.41	0	0.05	0
Kidney	<i>P. antarctica</i> ^c	2.02±1.47	0	0.35±0.11	0	9.30±2.06	0	1,820.42±352.46	0	0.24±0.47	2	14.95±0.67	0	126.05±25.18	0	0.67±0.15	0	30.61±2.42	0	27.54±14.47	0	0.15±0.06	1
	<i>P. adeliae</i> ^d	0.55±0.11	0	0.19±0.021	0	11.55±4.55	0	1,405.87±822.80	0	0.0004 ^a	2	15.34±1.87	0	141.75±4.21	0	0.33±0.04	0	8.29±1.00	0	22.03±10.47	0	0.06±0.03	0
	<i>P. papua</i> ^b	2.13±0.62	0	0.24±0.003	0	6.40±3.07	0	824.59±275.66	0	0.002±0.003	2	13.99±2.91	0	93.14±42.13	0	0.67±0.41	0	14.01±4.19	0	11.37±14.10	0	0.07±0.03	1
	<i>P. antarctica</i> ^b	0.69±0.38	0	0.24±0.12	0	10.13±2.37	0	362.83±75.61	0	0.0004 ^a	2	17.13±2.63	0	107.79±23.57	0	0.52±0.60	0	14.32±7.58	0	0.49±0.32	0	0.0008 ^a	2
	<i>P. adeliae</i> ^b	0.74	0	0.21	0	3.78	0	531.94	0	0.0004 ^a	1	10.74	0	163.71	0	1.07	0	19.30	0	54.41	0	0.0008 ^a	1
	<i>P. antarctica</i> ^c	0.75±0.76	0	0.33±0.37	0	5.35±0.75	0	669.58±224.13	0	0.09±0.17	3	15.33±4.67	0	149.85±49.23	0	0.58±0.12	0	69.88±19.66	0	263.93±139.77	0	0.18±0.01	1
Muscle	<i>P. adeliae</i> ^d	14.12±3.86	0	0.18±0.09	0	5.77±1.36	0	652.69±6.36	0	0.0004 ^a	2	14.78±3.04	0	234.30±62.24	0	0.38±0.12	0	30.23±6.03	0	351.84±0.08	0	0.21±0.17	0
	<i>P. papua</i> ^b	1.39±0.95	0	0.29±0.26	0	0.85±0.39	0	486.14±209.63	0	0.0004 ^a	3	7.97±1.15	0	103.07±60.55	0	0.63±0.53	0	3.17±0.53	0	0.11±0.18	0	0.18±0.05	1
	<i>P. antarctica</i> ^b	1.07±0.36	0	0.209±0.02	0	0.87±0.28	0	220.16±62.04	0	0.02±0.01	0	8.07±1.28	0	139.91±40.94	0	0.57±0.62	0	3.57±0.17	0	0.01±0.001	0	0.0008 ^a	2
	<i>P. adeliae</i> ^b	3.27	0	0.38	0	0.63	0	423.43	0	0.02	0	7.43	0	149.95	0	0.37	0	12.65	0	1.09	0	0.0008 ^a	1
	<i>P. antarctica</i> ^c	12.32±10.04	0	1.86±2.46	0	1.17±0.68	0	480.71±112.70	0	1.17±2.29	0	6.69±1.73	0	118.78±40.73	0	1.04±0.27	0	10.32±3.04	0	1.83±0.63	0	0.17±0.08	0
	<i>P. adeliae</i> ^d	1.78±1.91	0	0.46±0.22	0	1.11±0.39	0	595.83±264.46	0	0.0004 ^a	2	8.53±2.41	0	66.26±57.77	0	0.30±0.16	0	4.62±0.13	0	2.63±2.09	0	0.15±0.11	0
Bone	<i>P. papua</i> ^b	7.59±0.20	0	0.13±0.11	0	8.27±1.78	0	70.03±39.96	0	3.54±1.21	0	0.20±0.11	0	180.05±29.40	0	0.06±0.01	0	0.38±0.04	0	0.002±0.002	1	0.15±0.19	0
	<i>P. antarctica</i> ^b	4.16±1.02	0	0.16±0.07	0	6.66±0.57	0	44.08±33.04	0	0.25±0.01	0	0.17±0.22	0	221.29±9.19	0	0.08±0.07	0	0.73±0.29	0	0.0001 ^a	2	0.0008 ^a	2
	<i>P. adeliae</i> ^b	8.49	0	0.10	0	7.44	0	44.12	0	3.18	0	0.06	0	138.38	0	0.07	0	0.41	0	0.03	0	0.0008 ^a	1
	<i>P. antarctica</i> ^c	7.30±6.09	0	0.24±0.48	3	8.40±1.25	0	56.06±28.85	0	2.90±1.02	0	0.19±0.10	0	138.77±20.63	0	0.04±0.01	0	1.13±0.69	0	0.07±0.03	0	0.21±0.12	0
	<i>P. adeliae</i> ^d	5.61	0	0.0002 ^a	1	7.56	0	12.58	0	0.75	0	0.17	0	106.15	0	0.12	0	0.24	0	0.17	0	0.10	0
	<i>P. papua</i> ^b	6.71±5.15	0	0.68±0.03	0	0.06±0.09	1	16.61±0.05	0	0.17±0.14	0	19.26±0.94	0	69.49±6.32	0	0.10±0.05	0	2.41±0.41	0	0.02±0.004	0	0.33±0.31	0
Feather	<i>P. antarctica</i> ^b	7.99±10.02	0	0.09±0.05	0	0.21±0.30	1	29.74±29.14	0	0.05±0.01	0	15.29±0.34	0	94.75±2.37	0	0.31±0.21	0	4.33±0.01	0	0.01±0.01	1	0.02±0.03	1
	<i>P. adeliae</i> ^b	3.56	0	0.97	0	0.21	0	15.28	0	0.05	0	16.21	0	70.41	0	0.04	0	6.05	0	0.12	0	0.0008 ^a	1
	<i>P. antarctica</i> ^c	8.99±7.86	0	0.60±0.15	0	0.05±0.03	0	20.22±12.75	0	0.06±0.03	0	19.60±1.70	0	62.29±20.01	0	0.07±0.01	0	7.97±0.85	0	0.31±0.22	0	0.81±0.84	0
	<i>P. adeliae</i> ^d	0.71±0.43	0	0.31±0.05	0	0.0004 ^a	2	4.29±0.47	0	0.02±0.03	1	16.22±0.51	0	60.59±2.02	0	0.06±0.001	0	4.68±1.71	0	0.08±0.01	0	0.06±0.09	1
	<i>P. papua</i> ^b	2,594.61±1,306.72	0	1.41±1.36	0	82.43±27.49	0	3,245.77±2,415.55	0	0.38±0.20	0	30.51±35.73	0	19.84±4.63	0	2.00±0.09	0	2.42±2.49	0	0.09±0.11	0	0.71±0.42	0
	<i>P. antarctica</i> ^b	641.07±255.18	0	1.93±1.06	0	9.33±4.97	0	1,032.65±665.79	0	0.16±0.04	0	51.07±49.14	0	49.39±8.30	0	1.44±1.83	0	3.87±1.40	0	0.17±0.06	0	0.03±0.04	1
Stomach cont.	<i>P. adeliae</i> ^b	349.72	0	0.17	0	6.64	0	372.12	0	0.02	0	4.85	0	26.57	0	0.47	0	4.09	0	0.45	0	0.07	0
	<i>P. antarctica</i> ^c	193.52	0	1.74	0	5.99	0	934.01	0	0.31	0	54.86	0	46.67	0	1.77	0	23.22	0	0.71	0	0.12	0
	<i>P. adeliae</i> ^d	46.80±54.31	0	0.06±0.01	0	2.20±0.11	0	80.77±81.01	0	0.14±0.19	0	66.42±34.43	0	38.99±14.05	0	3.22±0.06	0	8.78±0.22	0	1.10±0.80	0	0.28±0.19	0

Data shown are mean±standard deviation

n number of non-detectable levels

^a Detection limit value

^b Location: King George Island

^c Location: Deception Island

^d Location: Avian Island

Table 4 Concentrations of trace elements (micrograms per gram dry weight) in juvenile Antarctic penguins

Samples	Species (Location)	Al	n	Cr	n	Mn	n	Fe	n	Ni	n	Cu	n	Zn	n	As	n	Se	n	Cd	n	Pb	n
Liver	<i>P. papua</i> ^b	1.62±1.00	0	0.49±0.45	0	8.30±0.57	0	6,246.20±2,941.77	0	0.19±0.04	1	386.13±174.48	0	237.19±22.38	0	0.79±0.63	0	10.69±0.76	0	0.40±0.18	0	0.0008 ^a	4
	<i>P. adeliae</i> ^b	1.93	0	0.08	0	15.83	0	3,432.88	0	0.004	0	22.89	0	182.58	0	0.30	0	8.19	0	0.18	0	0.0008 ^a	1
Kidney	<i>P. papua</i> ^b	4.80±4.24	0	0.59±0.52	0	7.33±3.38	0	575.27±127.82	0	0.02±0.02	0	19.99±6.83	0	152.14±18.51	0	0.43±0.17	0	10.69±2.27	0	1.54±0.71	0	0.0008 ^a	4
	<i>P. adeliae</i> ^b	3.48	0	0.29	0	7.79	0	573.02	0	0.01	0	12.66	0	119.90	0	0.45	0	15.09	0	0.68	0	0.0008 ^a	1
Muscle	<i>P. papua</i> ^b	2.01±1.96	0	0.50±0.03	0	0.52±0.06	0	271.74±75.67	0	0.01±0.004	1	9.95±2.08	0	139.39±46.68	0	0.36±0.21	0	2.61±0.31	0	0.01±0.01	0	0.0008 ^a	4
	<i>P. adeliae</i> ^b	3.41	0	0.20	0	0.91	0	156.63	0	0.0004 ^a	1	6.97	0	163.75	0	0.18	0	1.79	0	0.0001 ^a	1	0.0008 ^a	1
Feather	<i>P. papua</i> ^b	15.72±19.24	0	0.50±0.16	0	0.27±0.35	0	24.95±16.54	0	0.07±0.07	0	16.02±5.40	0	119.72±21.81	0	0.09±0.07	0	2.77±0.55	0	0.02±0.01	0	0.07±0.13	3
	<i>P. adeliae</i> ^b	52.44	0	0.11	0	1.15	0	21.92	0	0.0004 ^a	1	19.29	0	83.90	0	0.08	0	3.32	0	0.01	0	0.0008 ^a	1
Stomach content	<i>P. papua</i> ^b	854.88±1,000.14	0	3.03±4.89	0	16.27±15.69	0	2,884.27±1,466.78	0	0.30±0.30	0	7.33±1.13	0	41.09±16.40	0	0.28±0.07	0	2.86±0.84	0	0.12±0.07	0	0.05±0.02	1

Data shown are mean±standard deviation

n number of non-detectable levels

^a Detection limit value^b Location: King George Island

Unlike Se, we observed that Zn levels in liver and feather were higher in juveniles than in adult penguins ($p<0.05$). This pattern was also observed for Fe and Cu in penguins' liver ($p<0.05$) and can be related to the major requirements of these metals in young organisms (Mas 1993).

We found the highest Mn levels in the liver, kidney, and bone ($p<0.0001$; Tables 3 and 4) although our results suggest that Mn does not increase with age in penguins as occurs in other seabirds (Barbieri et al. 2010). In the last years, a potential increase of the environmental levels of Mn has been suggested to be due to the use of this metal as additive to combustibles (e.g. Burger and Gochfeld 2000; Mispagel et al., 2003). In this way, we found Mn levels in our samples slightly higher (between 0.87 and 2.13 times in hepatic tissue) than those detected by Honda et al. (1986) and Szefer et al. (1993b) two decades ago in tissues of adult Antarctic penguins (6.80–8.80 $\mu\text{g g}^{-1}$ d.w.) and similar to those detected more recently in penguin chicks by Smichowski et al. (2006) (10.00, 9.40, and 1.50 $\mu\text{g g}^{-1}$ d.w. in the liver, kidney, and muscle, respectively). Mn levels detected in our samples of Antarctic penguins were similar or slightly lower than those detected in liver, muscle or feather samples of Arctic seabirds (ranging from 7.13 to 15.00 $\mu\text{g g}^{-1}$ d.w. in liver, from 1.84 to 2.56 $\mu\text{g g}^{-1}$ d.w. in muscle and from 0.75 to 1.10 $\mu\text{g g}^{-1}$ d.w. in feather, see Burger et al. 2008 and Campbell et al. 2005) but sharply lower than Mn levels recently detected in feathers of adult seabirds coming from more industrialized and populated areas such as the Brazilian coasts (11.36 $\mu\text{g g}^{-1}$ d.w.; Barbieri et al. 2010). Although the comparison of metal levels among different species and populations of seabirds must be taken with caution (Jerez et al. 2011) and data are still scarce in Antarctica, our results seem to point out a certain increase of Mn pollution in Antarctica.

We observed differences in Pb concentrations in soft tissues (the liver, kidney, and muscle) and feathers between juvenile and adult penguins ($p<0.0001$ and $p<0.05$, respectively), which show that penguins accumulated Pb during their life time (see Tables 3 and 4). This accumulation occurred despite Pb can be partially eliminated during the process of feather growth (Jerez et al. 2011). The affinity of Pb to calcium-formations such as feathers brings about the accumulation of this toxic and non-essential metal in feathers during their growth and allows the elimination of an important proportion of Pb from the birds' body. In accordance with this, in juvenile specimens, we found detectable levels of Pb only in feather samples while internal tissues showed non-detectable levels (see Table 4). With respect to adult specimens, we also found the maximum level of Pb in feather samples (1.74 $\mu\text{g g}^{-1}$ d.w.) (see Table 3). So penguin feathers seem to be useful samples for the study of Pb exposure in these organisms, as well as for

monitoring the presence of this metal in the Antarctic environment.

Pb monitoring is often used to evaluate the presence of anthropogenic pollution in the environment (e.g., Sun and Xie, 2001), since this metal is not metabolically regulated for organisms (Gochfeld et al. 1996) and is emitted by several human activities. Some of these activities are currently carried out in Antarctica or have been carried out in the past (fuel combustion, waste incineration, sewage disposal, paint or accidental oil spills, among others; see Bargagli 2008 and dos Santos et al. 2005). Besides local activities, global environmental pollution can also increase Pb concentrations in Antarctica, and this heavy metal may find its way into the food web, bioaccumulate, and be passed along the food chain to penguins (Sun and Xie 2001). We detected the maximum Pb levels in penguin feathers from King George and Deception Islands (0.55 and 1.74 $\mu\text{g g}^{-1}$ d.w., respectively) where a relevant human presence exists. These results were consistent with those previously found (Jerez et al. 2011). These relatively high Pb levels detected in King George and Deception Islands were even comparable with Pb levels detected in seabirds' feathers from the Northern Hemisphere (Burger et al. 2008; Ribeiro et al. 2009) and seem to be influenced by anthropogenic sources of pollution. These results are in agreement with Sun and Xie (2001) who proved that Pb concentrations in Antarctic penguin droppings have increased during the last 200 years, especially in the last 50 years, as a consequence of the increasing global pollution and local pressure.

Ni tended to accumulate mainly in penguin bones ($p < 0.0001$, Table 3) where this metal reached levels significantly higher than those detected in penguins' stomach contents ($p < 0.0001$). Ni could also have been accumulated in penguin bodies during their life cycle since the concentration in muscle tissue was higher in adults than in juveniles ($p < 0.05$; see Tables 3 and 4). These results are indicative of the existence of Ni accumulation and magnification. Ni is an essential metal that is naturally distributed in the environment (Eisler 1998), although anthropogenic sources can increase natural Ni levels (e.g., mining, chemical industry, fuel combustion, waste incineration, sewage disposal, paint or accidental oil spills; ATSDR 2005). The limited availability of samples did not allow analyzing geographical differences in Ni concentrations in this study, but as in the case of Pb, we found the highest Ni concentrations in samples from King George and Deception Islands (bones—4.40 and 3.82 $\mu\text{g g}^{-1}$ d.w., respectively). These results suggest that anthropogenic sources could be responsible, at least partially, of Ni accumulation in penguins. Ni showed a high affinity to bone samples, and this kind of samples can be easily collected for Ni monitoring in the Antarctic environment since penguin bones are really abundant in rookery areas.

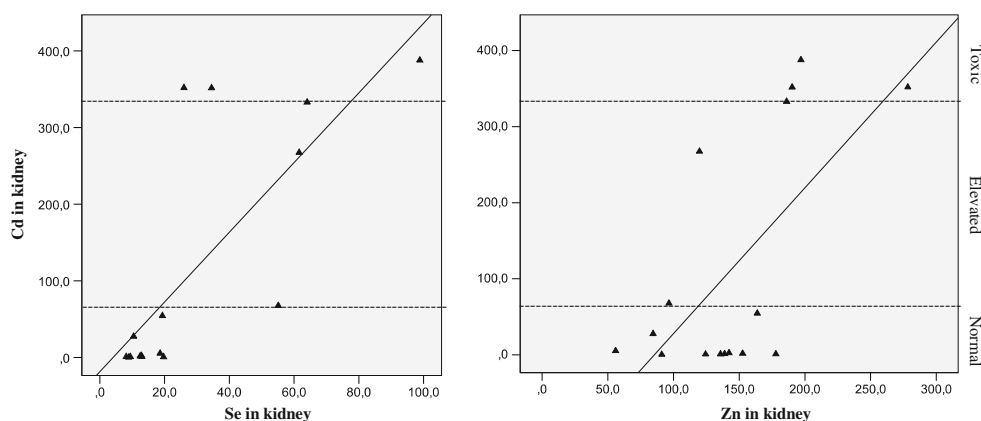
The highest Fe levels were found in soft tissues ($p < 0.0001$; Tables 3 and 4), especially in liver which has been described as storing tissue of Fe inside the organisms (Mas 1993). Fe levels were higher in hepatic tissues of penguins than in their stomach contents ($p < 0.05$).

Regarding differences among populations, Fe and Al levels seemed to be higher in stomach contents and feathers of penguins from King George and Deception Islands in comparison to penguins from Avian Island (see Table 3): Al and Fe in feathers were between 5.01–12.66 and 3.56–6.93 times higher, respectively, and Al and Fe in stomach contents were between 4.14–55.44 and 4.61–40.19 times higher, respectively. Unfortunately, these differences could not be statistically checked due to the sample shortage. The abundance of Al and Fe in samples from King George and Deception Islands could be related to the abundance of these metals in bioavailable forms in the sediments of these areas (Almendros et al. 1997; Deheyn et al. 2005; dos Santos et al. 2005; Rey et al. 1995).

The highest Cu levels were found in the liver, kidney, and feathers ($p < 0.0001$; Tables 3 and 4). Cu levels in liver of penguins were similar to those detected in other seabirds from the Northern Hemisphere (ranging from 19.10 to 92.50 $\mu\text{g g}^{-1}$ d.w., see Lucia et al. 2010 and Ribeiro et al. 2009). Penguin feathers showed Cu levels even higher than seabird feathers from China (4 $\mu\text{g g}^{-1}$ d.w., see Liu et al. 2006). These high Cu levels detected in penguins seem to be directly connected with the high Cu amounts detected in Antarctic krill in this study (maximum level, 90.76 $\mu\text{g g}^{-1}$ d.w.) and previously (41.30 $\mu\text{g g}^{-1}$ d.w.; Nygard et al. 2001). However, temporal trends on Cu levels in Antarctica should be checked in the future since human activities (oil spills, sewage, or solid waste) can contribute to increase Cu levels in coastal marine ecosystems (Eisler 1981).

The highest levels of As were found in soft tissues ($p < 0.0001$; Tables 3 and 4) where this metal is rapidly distributed and retained when goes into the body (ATSDR 2007). Our results on As levels in soft tissues were similar to those detected in chick penguins from King George Island (0.50, 0.55, and 0.82 $\mu\text{g g}^{-1}$ d.w. in the liver, kidney, and muscle, respectively, see Smichowski et al. 2006) and to those detected in Arctic seabirds (from 0.17 to 0.32 $\mu\text{g g}^{-1}$ d.w. in feather samples, see Burger et al. 2008). Seabirds from other regions of the world showed a wide range of As levels (from 0.22 to 5.62 $\mu\text{g g}^{-1}$ d.w. in liver; Lucia et al. 2010; Ribeiro et al. 2009) which could be related to differences in their diets (Kubota et al. 2001). In accordance with Braune and Noble (2009), As levels in our samples were lower than 3 $\mu\text{g g}^{-1}$ d.w. (see Table 3), which are usual levels in living organisms. The As values reported in this study were very low in comparison to values known to produce toxic effects, such as endocrine disruption, in seabirds ($>50 \mu\text{g g}^{-1}$ d.w.; Neff 1997).

Fig. 2 Normal, elevated, and toxic Cd levels in penguin renal tissues and correlations with Se and Zn levels. Data are shown as micrograms per gram dry weight. Toxic threshold taken from Furness (1996)



Cr did not show a clear pattern of accumulation in any specific tissue, although we found the highest Cr levels in muscle tissue of specimens from King George and Deception Islands (maximum levels—2.10 and 5.52 $\mu\text{g g}^{-1}$ d.w., respectively). Unlike Szefer et al. (1993b) that found non-detectable Cr levels ($<0.03 \mu\text{g g}^{-1}$ d.w.) in the majority of the analyzed tissues of Antarctic penguins, we found detectable levels in the 95.45 % of them. These results seem to indicate that an increase on Cr levels can be occurring in Antarctica, although this possibility should be taken with caution since data on levels of this metal in penguins are still scarce and could be influenced by diet differences among populations. This possible increase could be related with the increase of human presence in some Antarctic areas such as King George or Deception Islands since Cr is associated with several human activities and oil contamination (Alam and Sadiq 1993; Caccia et al. 2003). The majority of the studied specimens of penguins showed Cr levels one order of magnitude lower than those detected in soft tissues of seabirds from the Northern Hemisphere, although feathers of penguins from King George and Deception Islands showed Cr levels close to those detected in feathers of Arctic seabirds (from 1.81 to 8.22 and from 0.95 to 2.03 $\mu\text{g g}^{-1}$ d.w. in soft tissues and feathers, respectively, see Burger et al. 2008 and Ribeiro et al. 2009).

Al, Cr, Mn, Cu, and As did not show signs of magnification or accumulation during the penguins' life time.

The 72.97 % of the observed correlations between pairs of elements were positive (Table 5). The predominance of this kind of correlations among the studied elements may suggest common uptake and storage pathways or similar regulation and detoxification processes in penguins as occur in other seabirds (Mendes et al. 2008; Nam et al. 2005; Perez-Lopez et al. 2006; Ribeiro et al. 2009).

Specifically in feathers, we found six different positive correlations between pairs of elements, suggesting that penguin feathers accumulate metals during their growth due to the existence of a high blood flow. This accumulation in

feathers allows the elimination of partial contents of toxic metals from the organism.

In soft tissues, we also found several positive correlations between essential elements such as Fe–Zn, Fe–Se, Fe–Cu, Fe–Ni, and Cu–Zn that can indicate the existence of similar metabolic regulations for these pairs of elements (Ribeiro et al. 2009). We found other positive correlations between pairs of toxic elements known to be related with the presence of contaminant activities (Cr–Mn, Cr–Pb, Mn–As, Mn–Pb, and Cd–Pb) suggesting the existence of common anthropogenic sources of these metals in the study area.

We also found negative correlations such as the observed between Pb–Zn, Pb–Cu, and Pb–Fe in liver. These results may be related to the ability of Pb to supplant polyvalent cations and use their transport mechanisms (Ballatori 2002), hindering the transport and accumulation of essential elements in the studied specimens.

Conclusions

The studied penguins, especially those specimens collected in Deception and Avian Islands, were chronically exposed to high Cd levels, and this metal was mainly accumulated in renal tissue. The study of Cd levels in penguin tissues and their stomach contents (composed mainly of krill) showed signs of biomagnification for this metal in the Antarctic food web. Several specimens of penguins showed elevated Cd levels in the kidney, even above the toxicity threshold proposed for birds. We also found high Se and Zn levels in penguin tissues which could be playing a protection role against the toxic effects of the elevated exposure to Cd. Se even reached toxic concentrations in hepatic tissue. The obtained results indicated that a slight increase in Mn and Cr levels in Antarctica could exist and could be related with a major human presence, use of combustibles, and oil contamination.

Feathers seem to be an important elimination way of the absorbed Pb in penguins. In spite of that, Pb was accumulated during the penguins' life cycle. Ni was also accumulated in

penguins, mainly in bones. Feathers and bones could be useful samples non-invasively and easily collected for monitoring Pb and Ni, respectively, in the Antarctic ecosystems. The highest levels of Pb and Ni in this study were detected in specimens from areas where a major human presence exists (King George and Deception Islands), and this fact suggests that these metals, at least partially, came from anthropogenic sources.

High Cu levels were detected in penguins probably related to the high Cu levels present in their main prey, Antarctic krill. Signs of bioaccumulation or biomagnification phenomena were not observed for Cu and neither for Al, Cr, Mn, and As in the studied specimens.

Finally, we found a wide number of positive correlations between pairs of elements. These results indicate in general that similar uptake and storage pathways existed for them, as well as similar internal processes of regulation and/or detoxification. And particularly for metals known to be related with anthropogenic contamination (Cr, Mn, As, Cd, Pb), these results suggest that common anthropogenic sources existed for them in the study area. These sources seem to be increasing the presence of toxic metals in Antarctic penguins.

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